

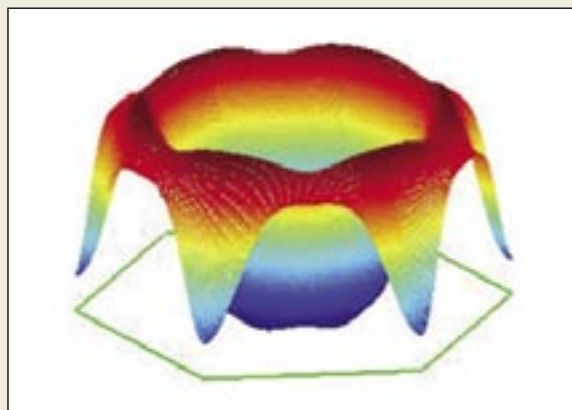
X-RAY ANALYSIS METHOD CRACKS THE 'LIPID PHASE PROBLEM'

Water-repellent lipids, such as fats and waxes, are a major class of molecules that form cell membranes and store energy, among other key functions. However, although these long chain molecules tend to form ordered crystal structures suitable for a common structural analysis method — x-ray diffraction — determining exactly how they pack together is difficult. This is due to a glitch in the diffraction method: the “phase problem.”

The phase problem exists because the diffraction pattern produced by the x-rays can only take into account the intensity of the x-rays used, not their phase (the particular position in time of the x-ray light waves). This limits the quality of the structural data that can be obtained. But recently at the NSLS, a research group learned how to use a common crystallographic method, called multi-wavelength anomalous dispersion (MAD), to determine the phases of diffraction from lipid structures. This important work may help elucidate many biological processes that involve lipids, particularly cell-to-cell fusion.

“Knowing just how lipid chains pack together is of considerable importance in biology,” said the study’s lead scientist, biophysicist Huey Huang of Rice University. “But the details of small-scale shape changes in these lipid structures are not well understood. We think that our work can help remedy this problem.”

Normally, how a molecular structure diffracts x-rays does not depend on the x-ray wavelength or energy. But this is not true when the structure contains atoms that have an absorption edge — a particular energy for which the x-rays are strongly absorbed — near the energy of the incident x-rays. These are called “anomalous” atoms. The MAD method makes use of this energy dependence to single out the contribution of the anomalous atoms in the overall diffraction behavior. This greatly simplifies the original phase problem. And once



A three-dimensional view of the electron density distribution of bromine atoms in the hexagonal array of lipid cylinders.

the phase problem is solved, the structure of the crystal can then be determined. MAD is routinely used to study protein structures, but, according to Huang and his group, this is the first time it has been used to solve a lipid crystal structure.

One difference between lipids and proteins is that lipid samples take the form of thin films deposited on a substrate, such as a silicon wafer. These thin-film samples often do not have a uniform thicknesses, a technical problem that makes the standard method of MAD analysis difficult. Huang and his group developed a new way to analyze the thin film samples.

The lipid molecules they investigated are members of a lipid sub-class called phospholipids, which are a major component of cell membranes. The particular phospholipids used here were “labeled” with a bromine atom. Together, the molecules pack together such that they form cylinders arranged in a hexagonal array.

The Huang group’s analysis shows that the lipid chains pack together uniformly with each chain occupying the same volume, rather than one or two chains taking up more or less space than the others. Lipid chain volume has been assumed to be constant under configuration changes, but this is the first direct experimental proof.

The group is hoping, in particular, to shed light on “membrane fusion,” one step in the process by which two cells join to become a single cell. The membranes meet at one location and create a connection between the cells, allowing the exchange of molecules (this, for example, is how fertilization and viral infection can occur). Eventually, the membranes merge to create one larger membrane enveloping the contents of both cells. During all of this, the lipid layers in each membrane undergo a series of structural changes. Now, thanks to the work of Huang and his colleagues, scientists may be able to identify those changes to better understand membrane fusion.

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For more information, see: D. Pan, W. Wang, W. Liu, L. Yang, and H. Huang, “Chain Packing in the Inverted Hexagonal Phase of Phospholipids: A Study by X-ray Anomalous Diffraction on Bromine-Labeled Chains,” *J. Am. Chem. Soc.*, **128**, 3800-3807 (2006).

— Laura Mgrdichian